

LMO and ALK

**The Unbelievable
Likeness of Beings**

Heberlein Lab

UCSF and EGCRG

Why flies ???

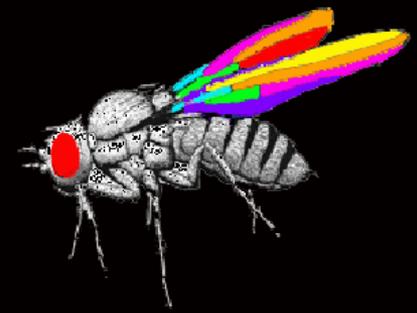
Because we believe:

- similarity of drug-induced behaviors
- conservation of synaptic machinery involved in drug responses
- conservation of genetic bases of drug-related behaviors

Because we can:

- awesome classical and molecular genetics
- economy of scale
- robust behaviors
- sophisticated tools to manipulate genome and neural circuits

The Approach



Develop behavioral model



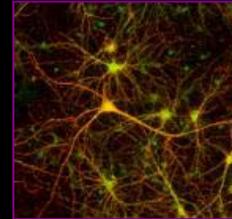
Genetic screens



Mutants



Genes



Candidate genes

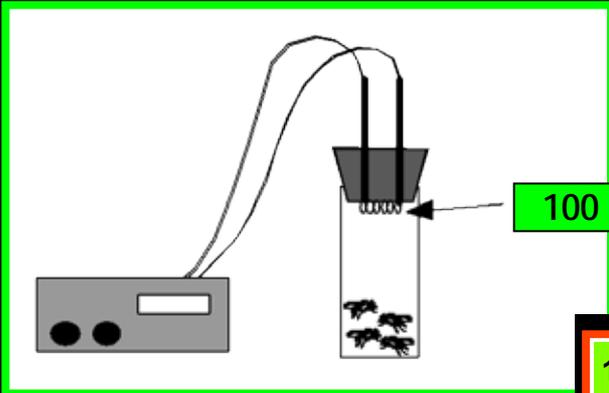
Mechanisms

Targets for pharmacotherapy



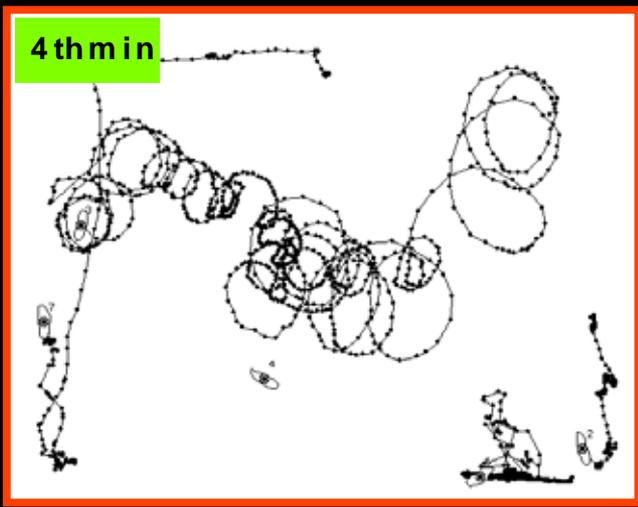
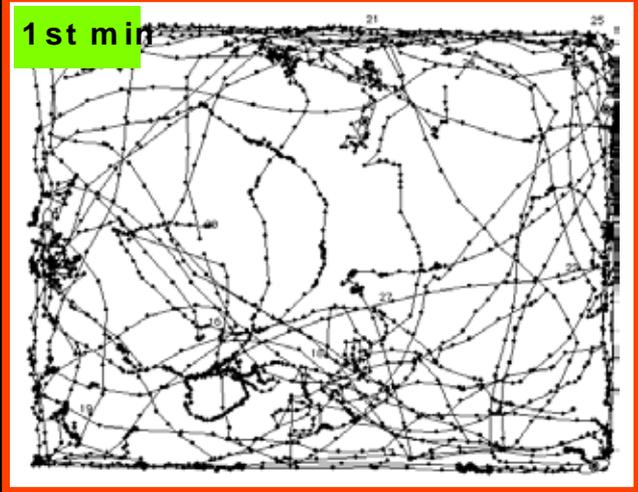


Acute cocaine exposure



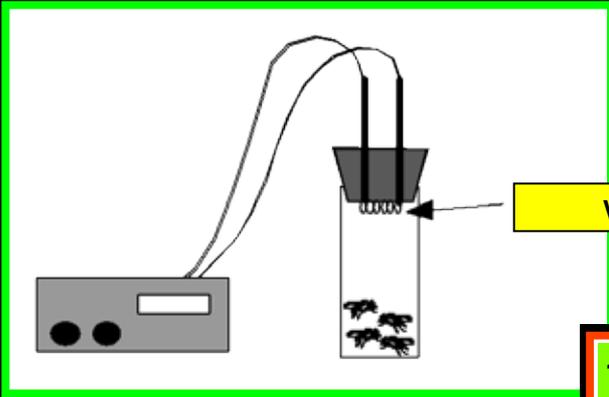
100 µg cocaine

McClung and Hirsh



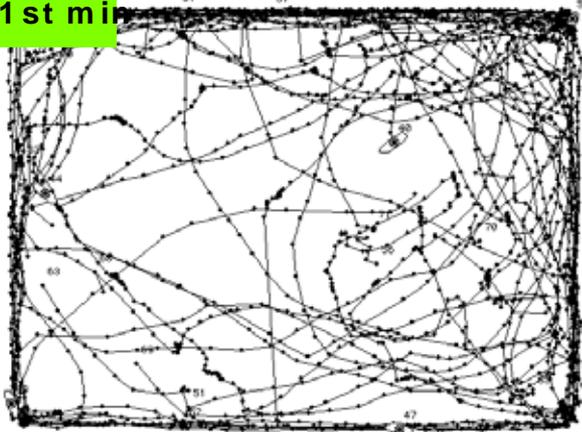
Linus Tsai

Mock exposure

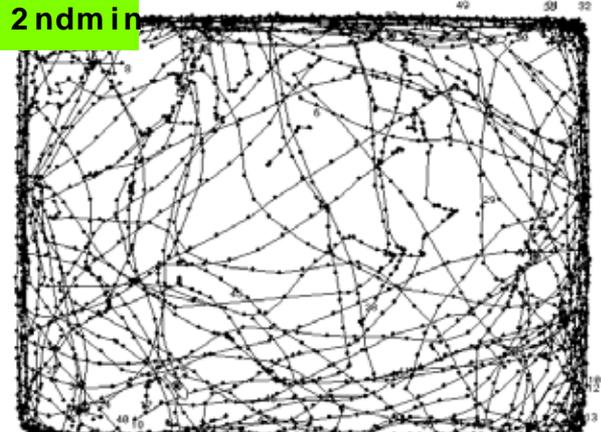


McClung and Hirsh

1st min



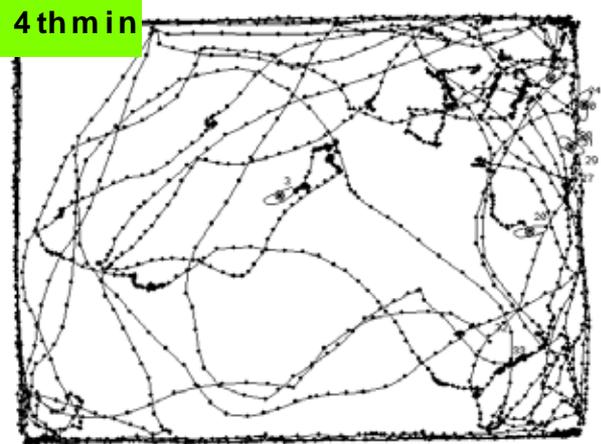
2nd min



3rd min



4th min

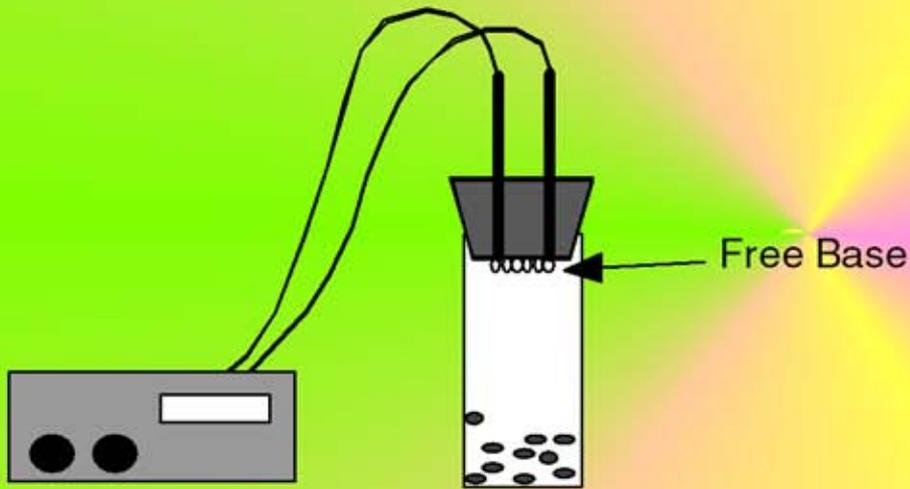


Linus Tsai

A simple behavioral assay



Cocaine / Nicotine Exposure



“Crackometer”



$$\frac{(2 \times 5) + (1 \times 1) + (0 \times 2)}{2 \times \text{total \# flies}} \times 100 = \text{BS, \%}$$

Cocaine Sensitivity Mutants

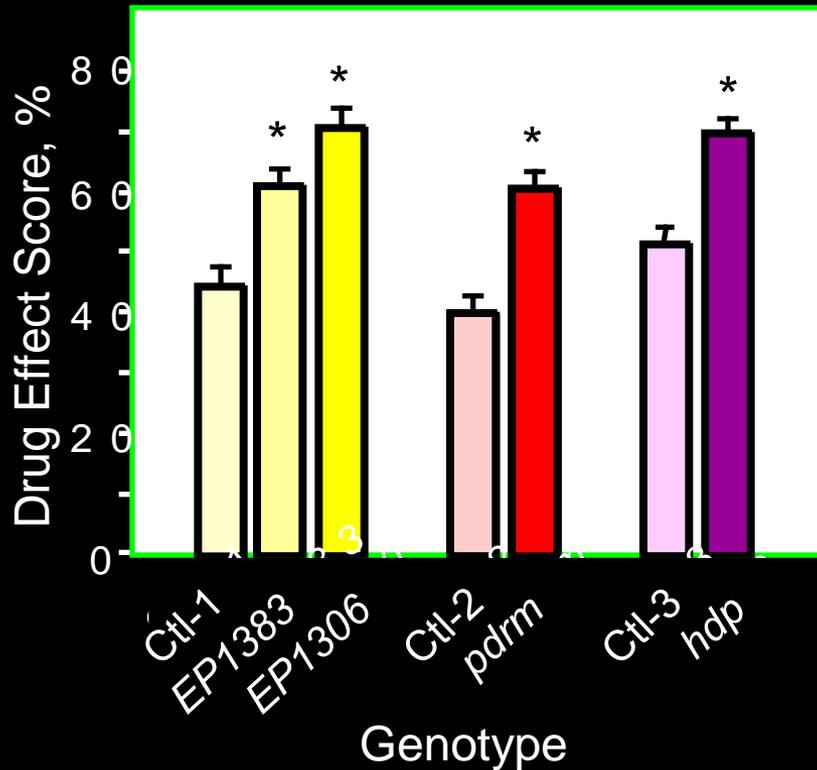
(of ~ 400 lines screened)

Line	Phenotype	Gene or homology
EP1a	RRR	
EP1b	RRR	RhoGAP18B (<i>white rabbit</i>)
EP1c	RR	
EP2	RRR	Alpha-mannosidase
EP3	RR	MAPKKK (<i>dtao</i>)
EP4a	S	Bx / LMO
EP4b	SS	Bx / LMO
EP5	SS	GPCR (<i>moody</i>)
EP6	SS	GSK3 (<i>shaggy</i>)
EP7	SS	mRNA binding protein
EP8	S	Neuropeptide (<i>amnesiac</i>)
EP9	S	<i>period</i>
EP10	S	Ethanolamine kinase (<i>easily shocked</i>)

Effect of *dLmo* mutations on cocaine sensitivity

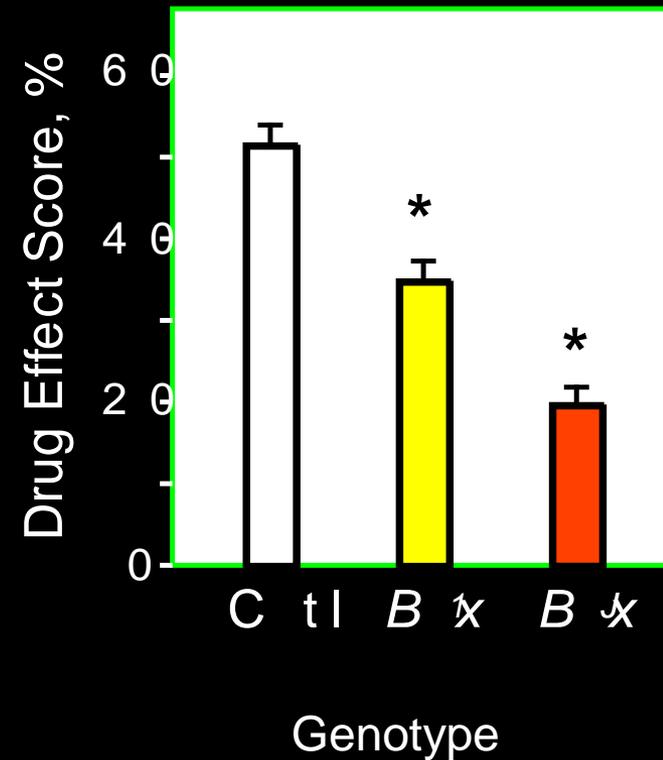


Loss-of-function



↓ *dLmo* expression in NS

Gain-of-function



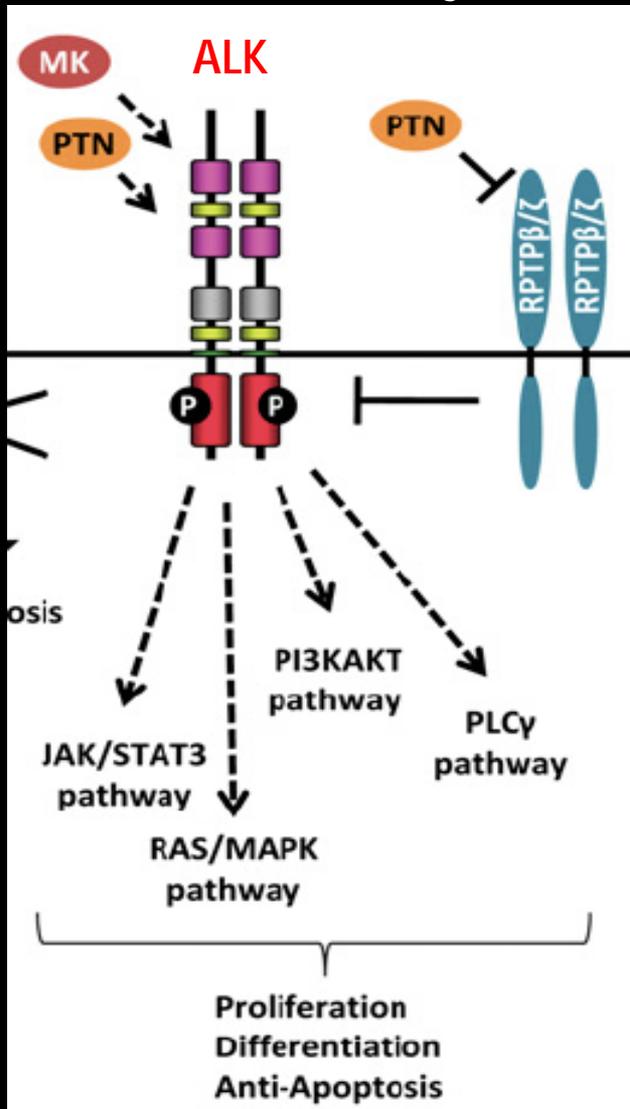
↑ *dLmo* expression in NS

Cocaine sensitivity is inversely proportional to [dLMO]

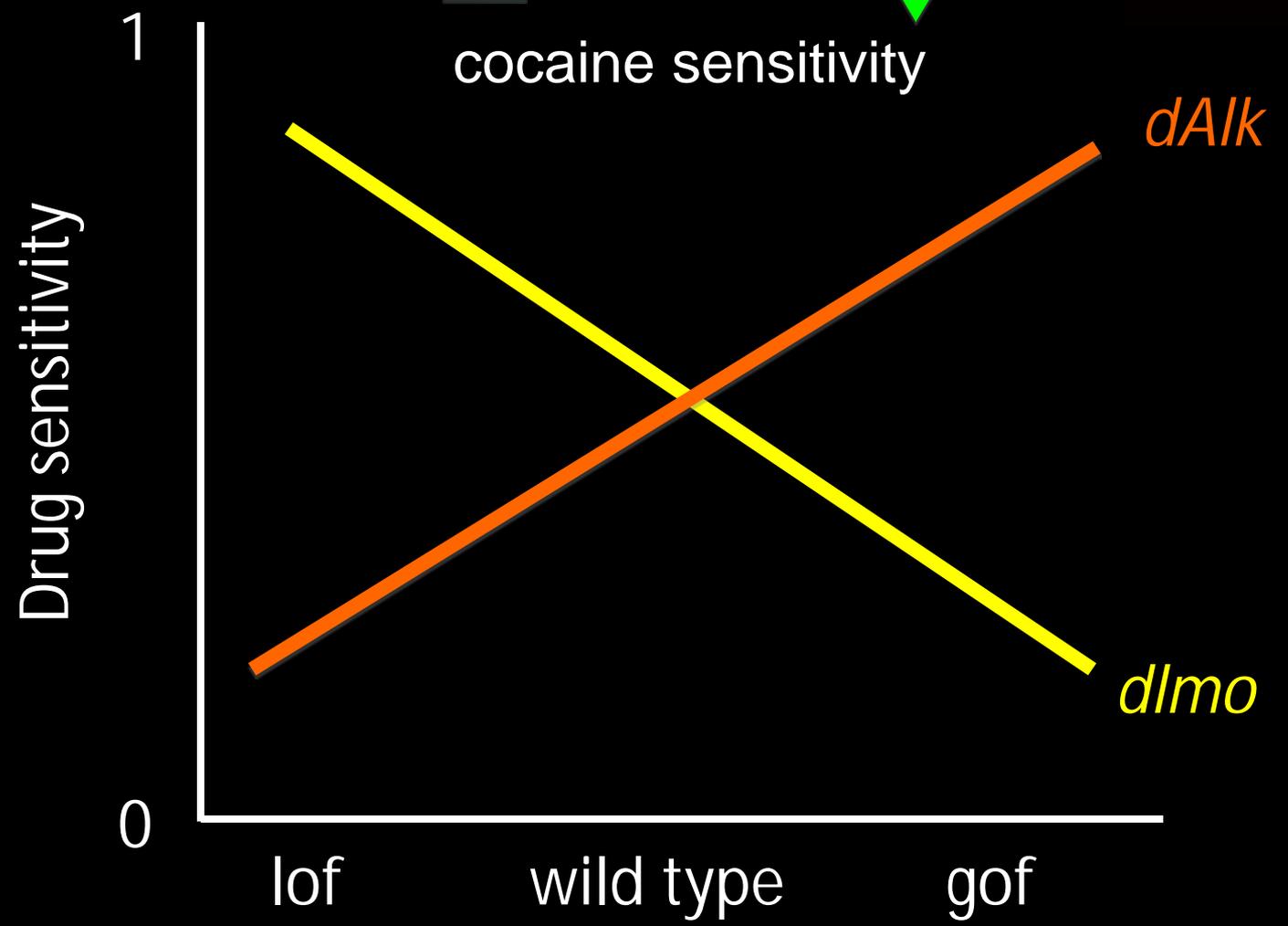
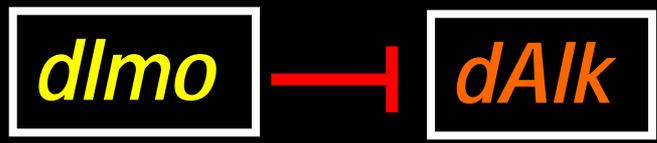


ALK: A Receptor Tyrosine Kinase

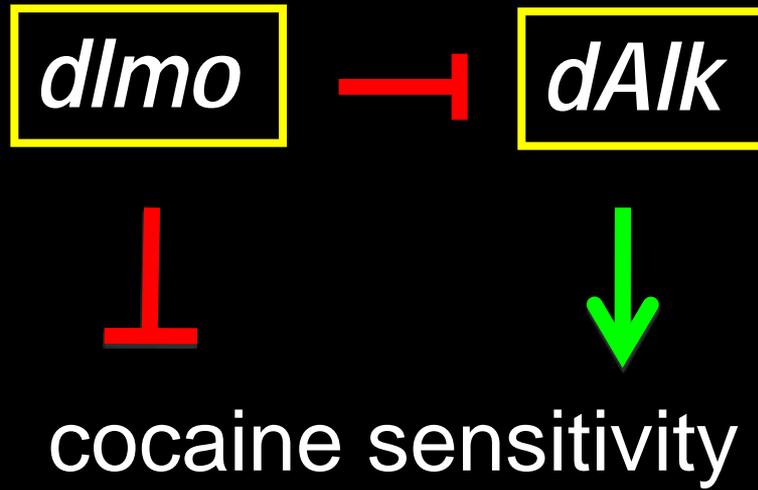
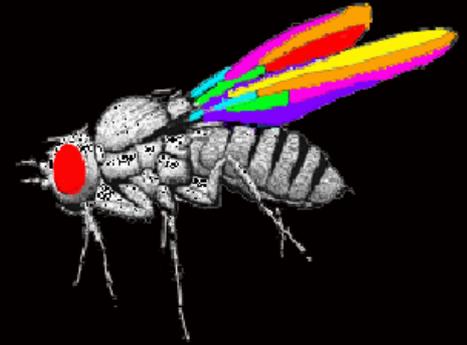
ALK Pathway

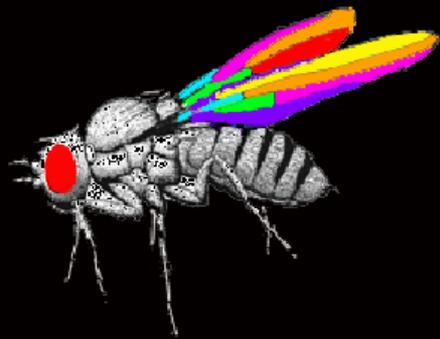


- **Oncogenic** when fused to other proteins through chrom. translocations (Morris et al, 1994)
- Activation of Alk promotes **neurite outgrowth** in culture (Souttou et al, 2001)
- Expressed primarily **in brain**, particularly during development (Iwahara et al, 1997; Vernersson et al, 2006)
- KO mice viable, exhibit **"antidepressant"** phenotype (Bilslund et al, 2008)

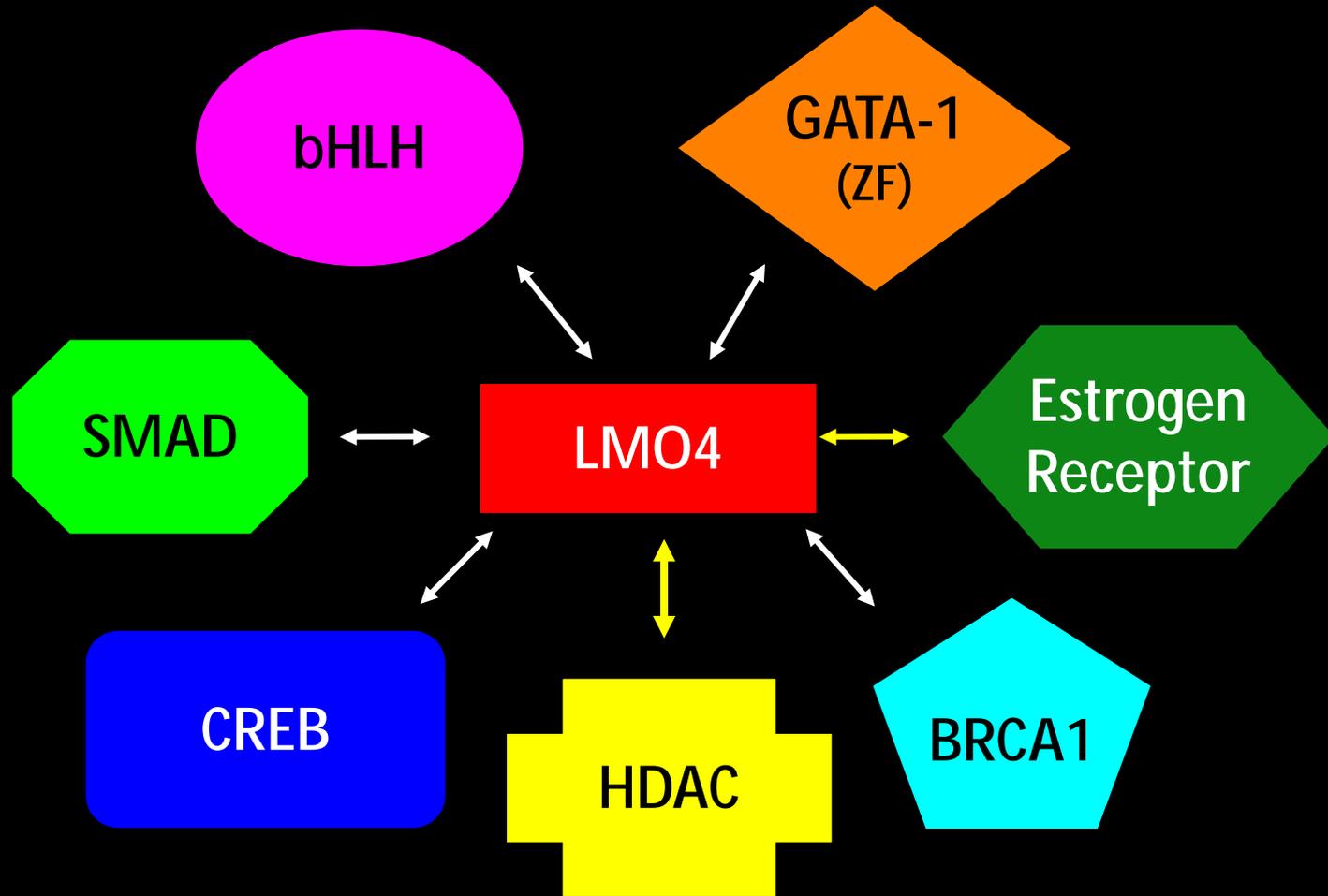


cocaine sensitivity



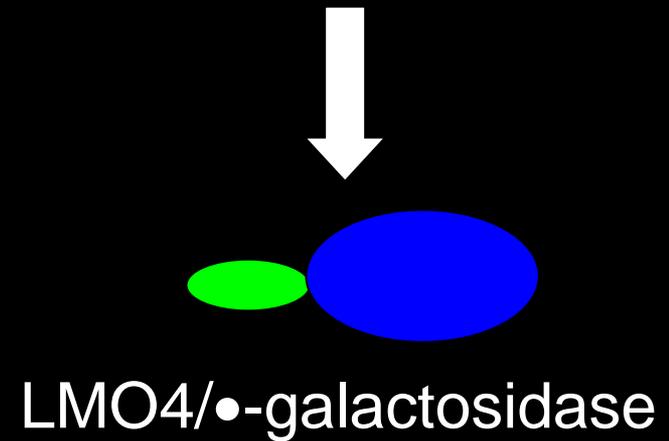
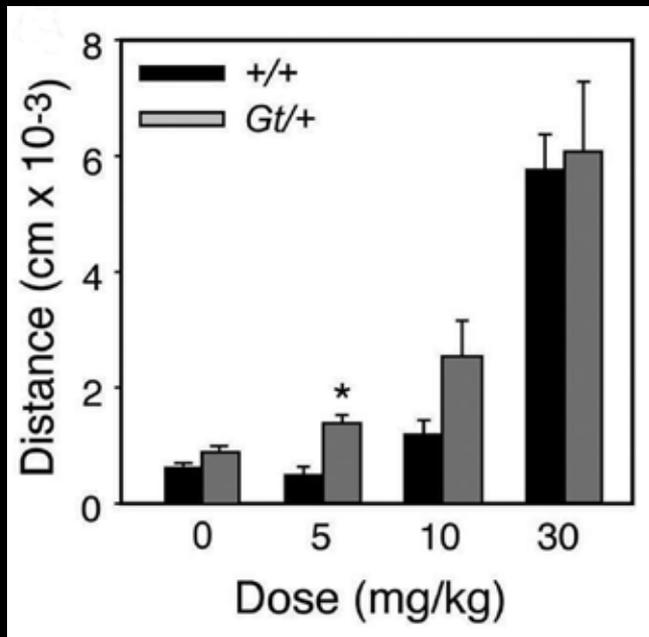
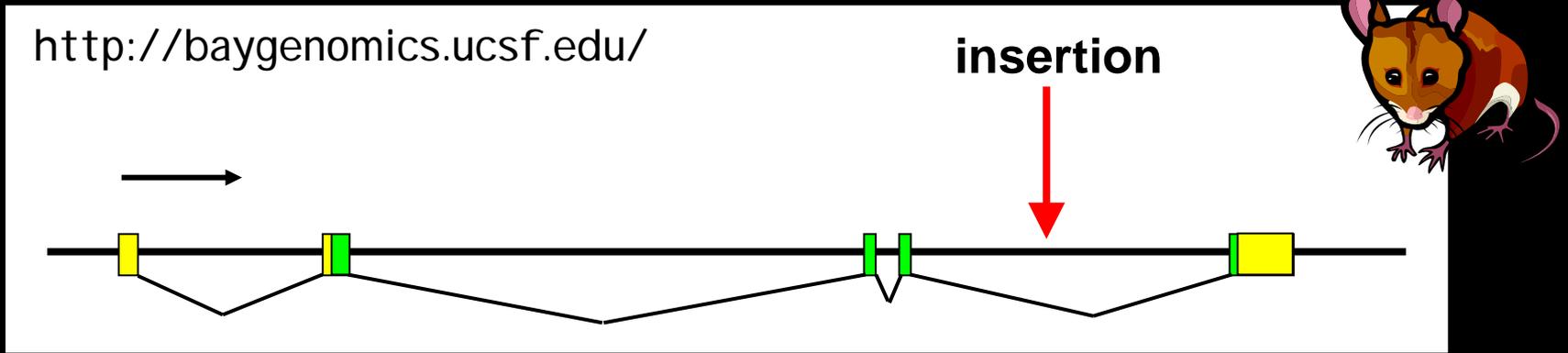


Mammalian LMOs interact with multiple partners



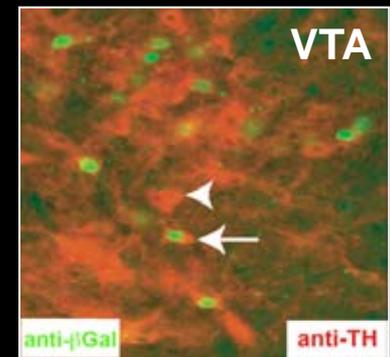
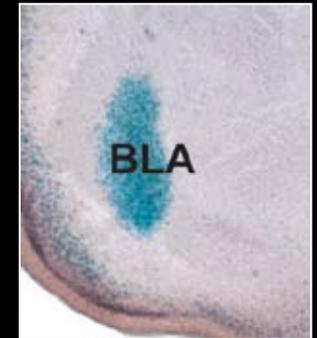
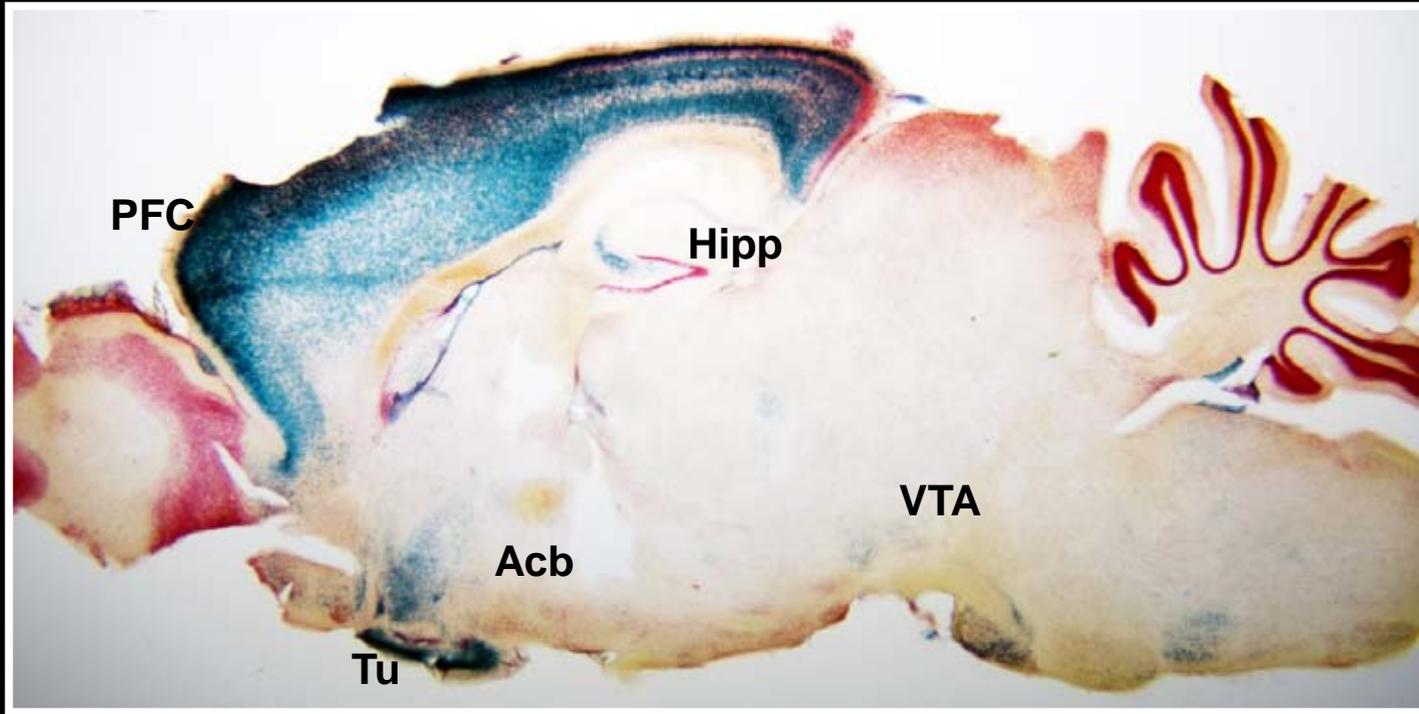
LMOs can act as repressors or activators of transcription complexes

A null allele of *Lmo4* generated from gene-trap ES cells

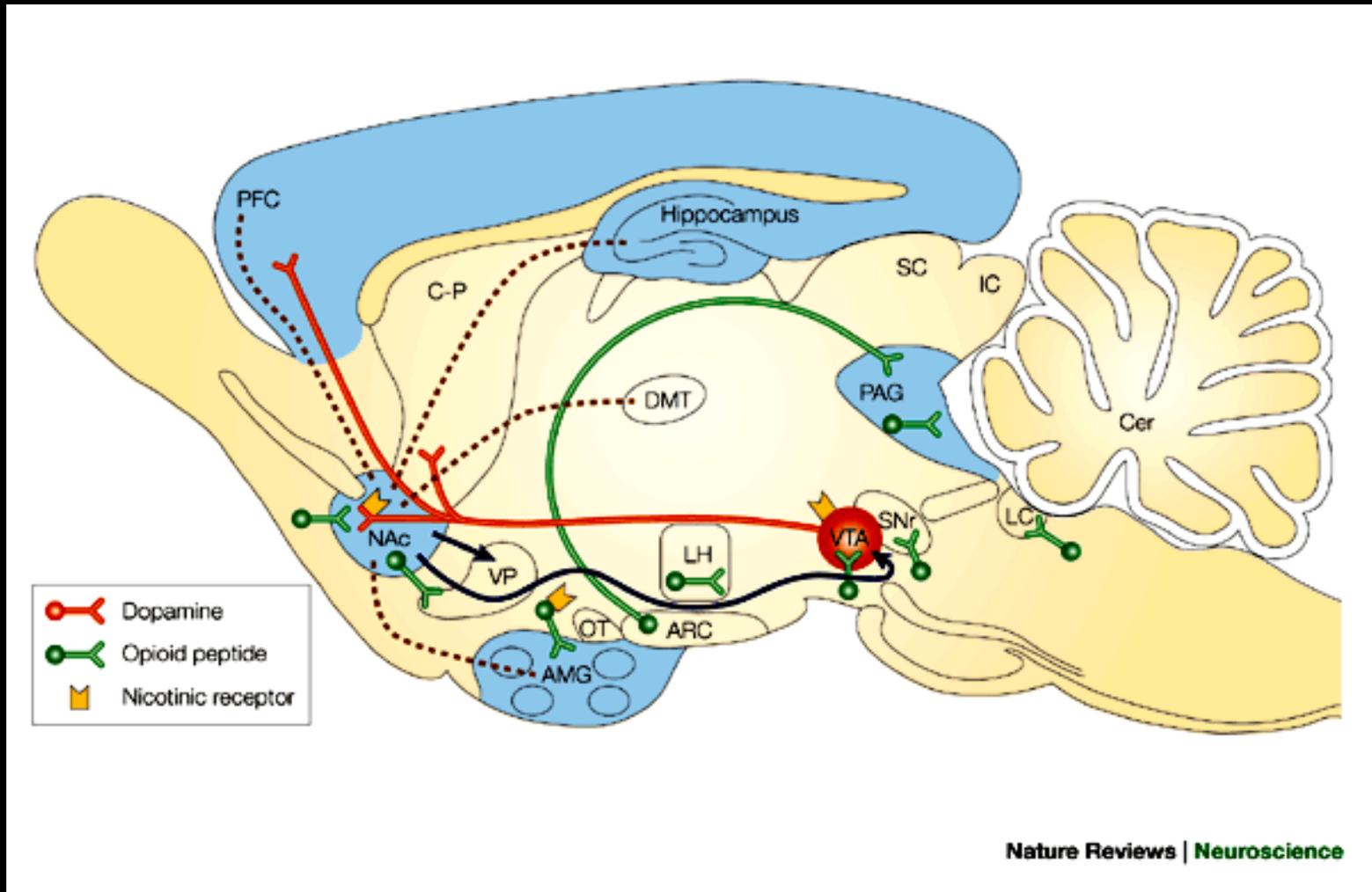


Heterozygous mice show enhanced cocaine sensitivity

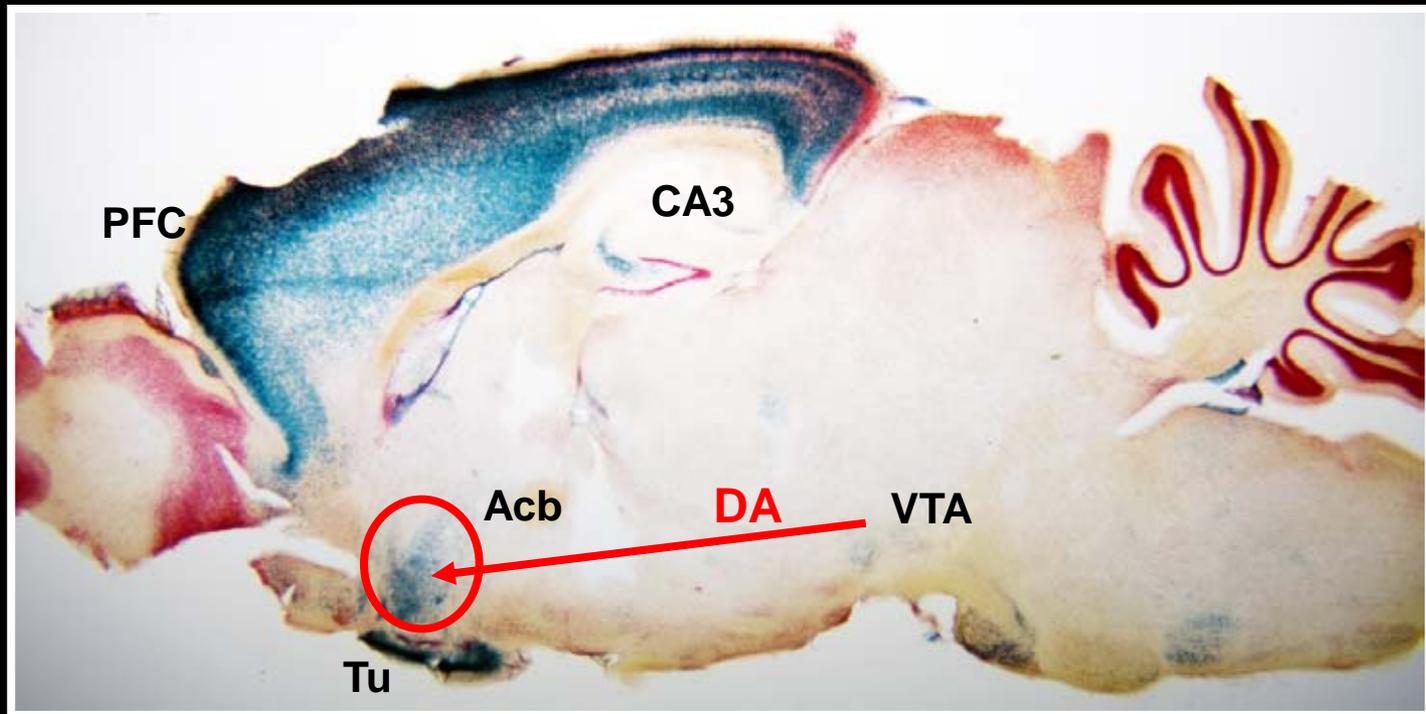
LMO4- β GAL fusion protein expression in adult *Lmo4^{gt/+}* mouse brain



Brain regions implicated in addiction-related behaviors

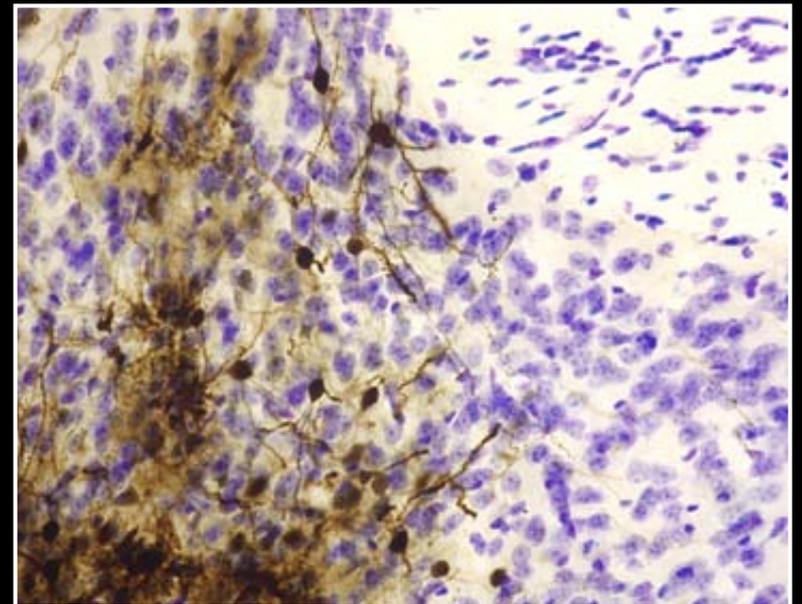
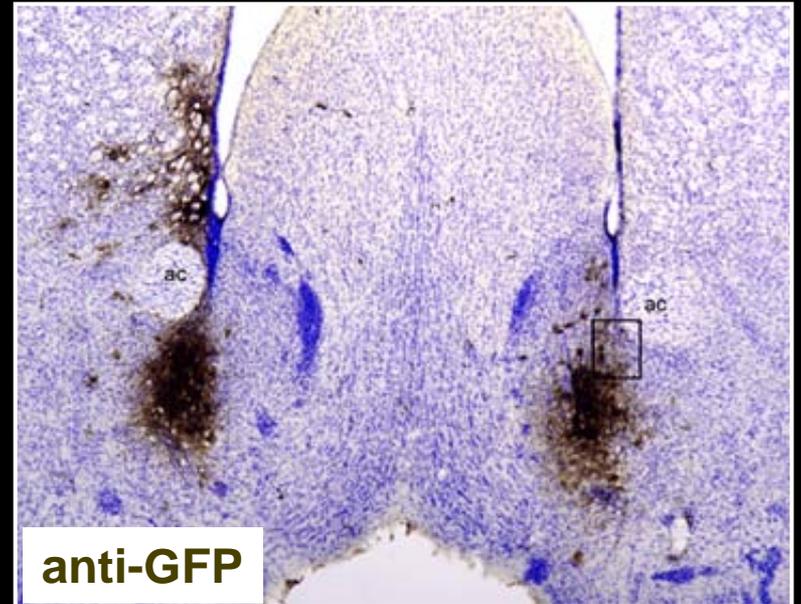


Where and **when** is *Lmo4* function required to regulate cocaine-related behaviors?

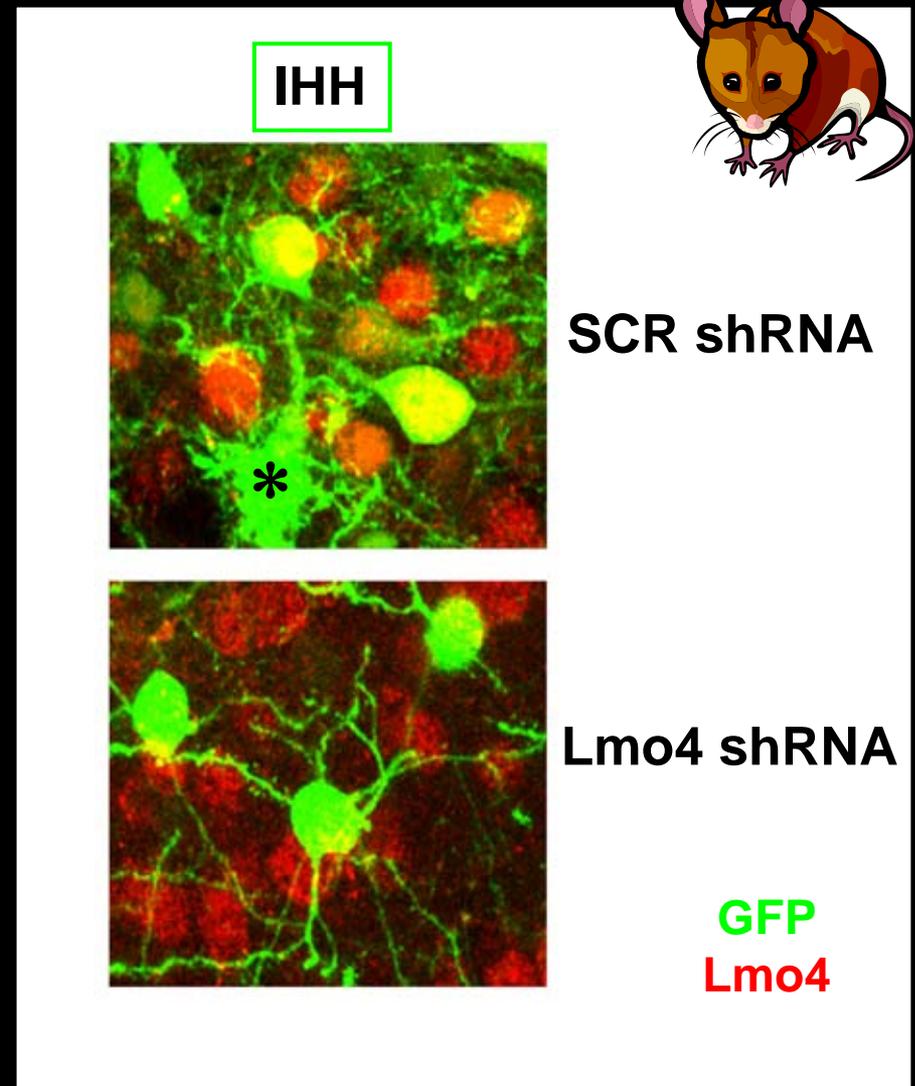
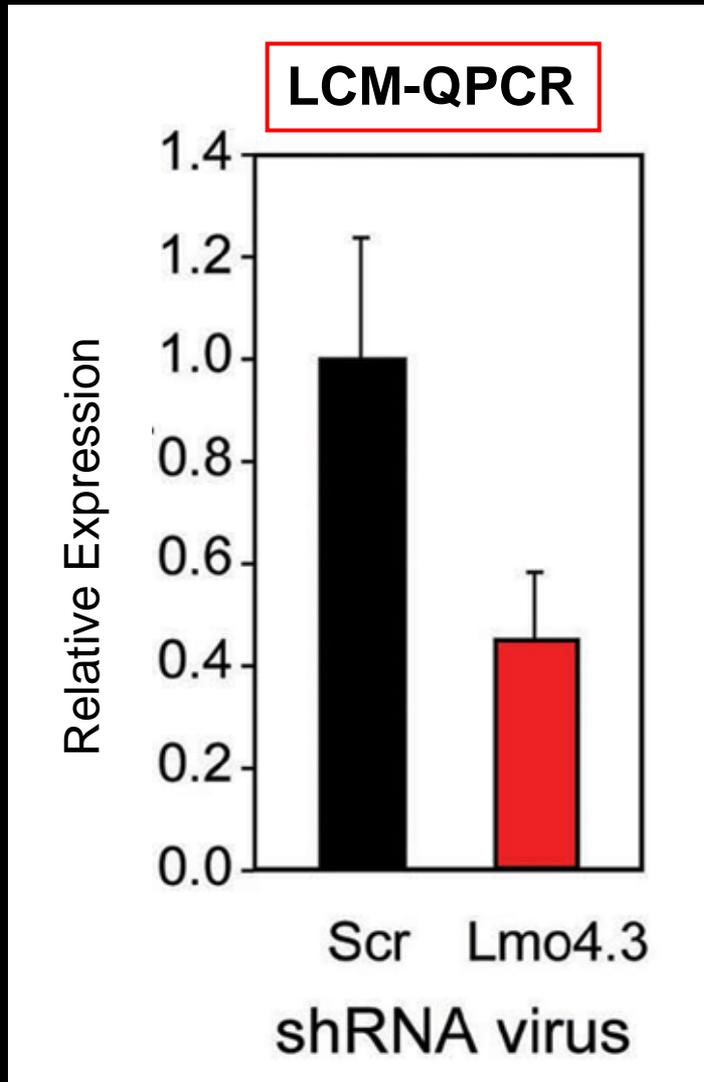


Virally-mediated RNA interference

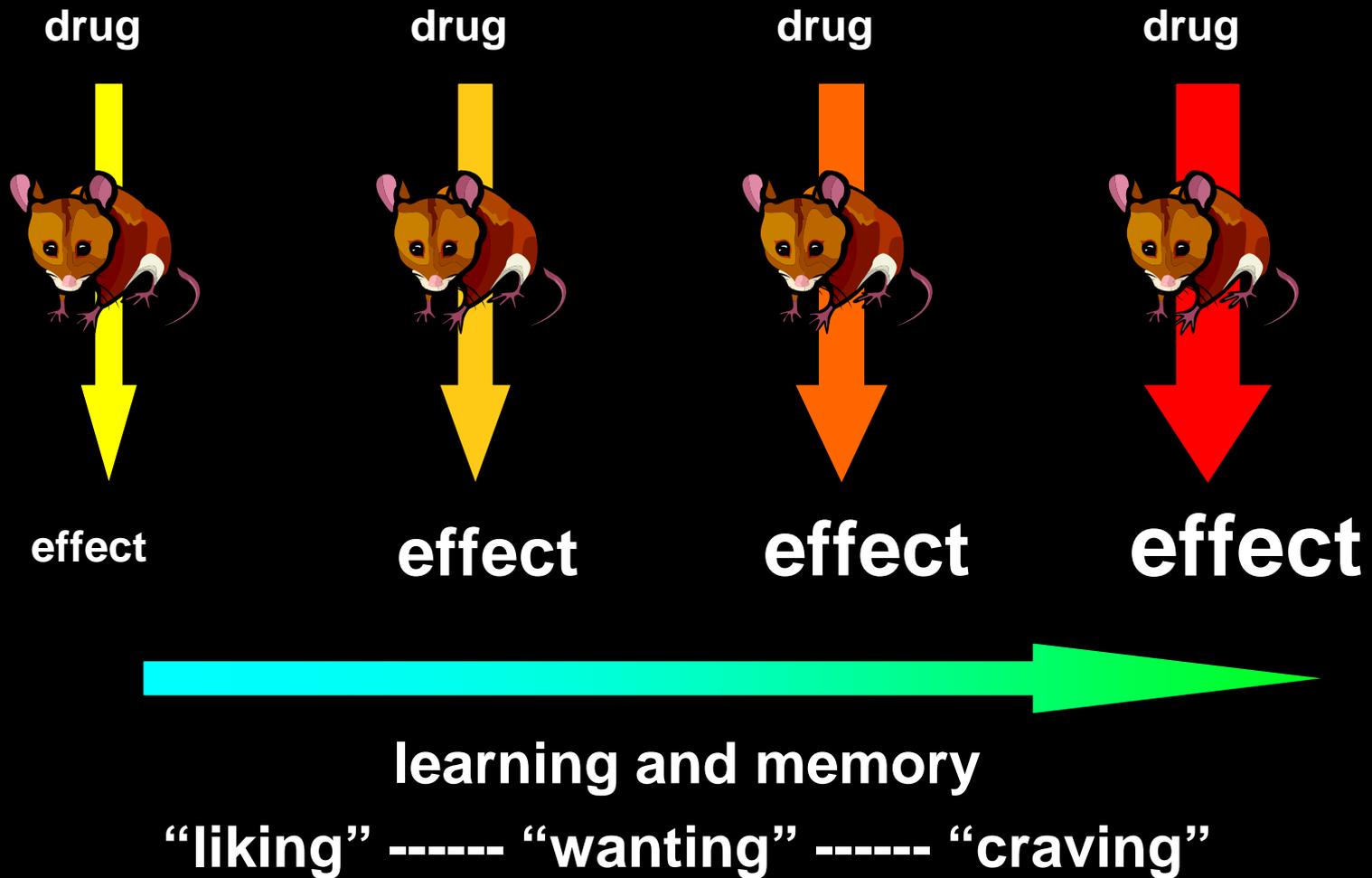
Viral delivery to and infection of Nucleus Accumbens



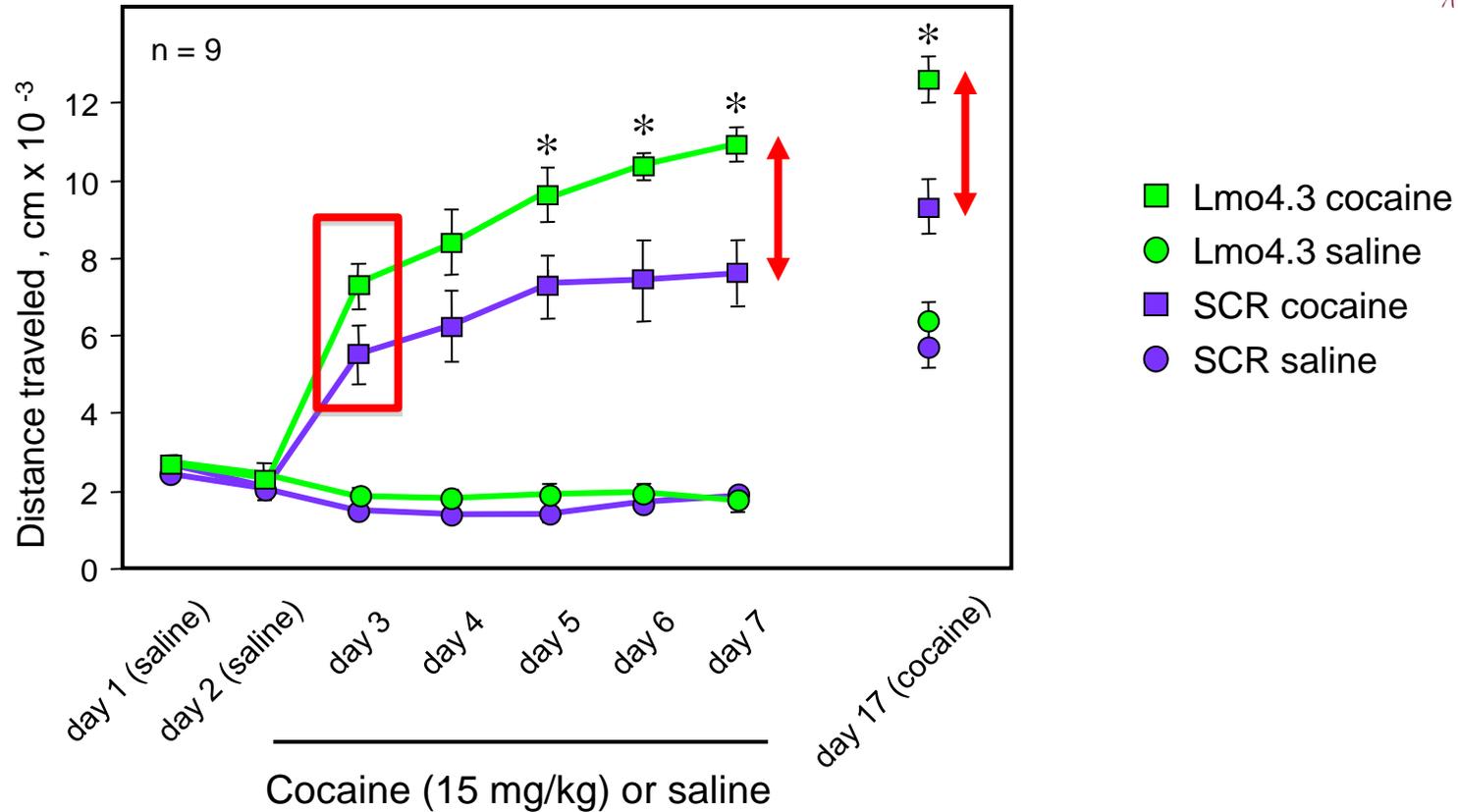
shRNA reduces *Lmo4* mRNA and protein in Acb



Sensitization: A long-lasting neuroadaptation induced by repeated drug exposure



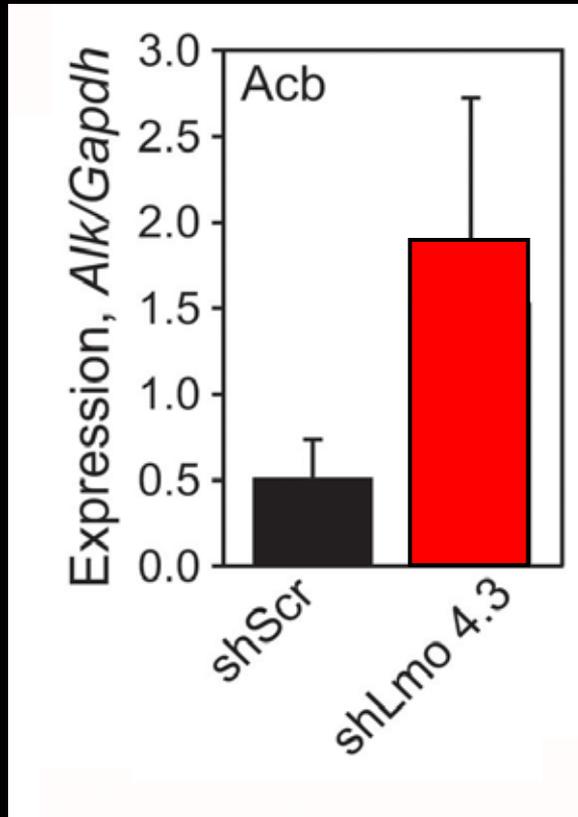
Cocaine sensitization enhanced by *Lmo4* RNAi in Acb



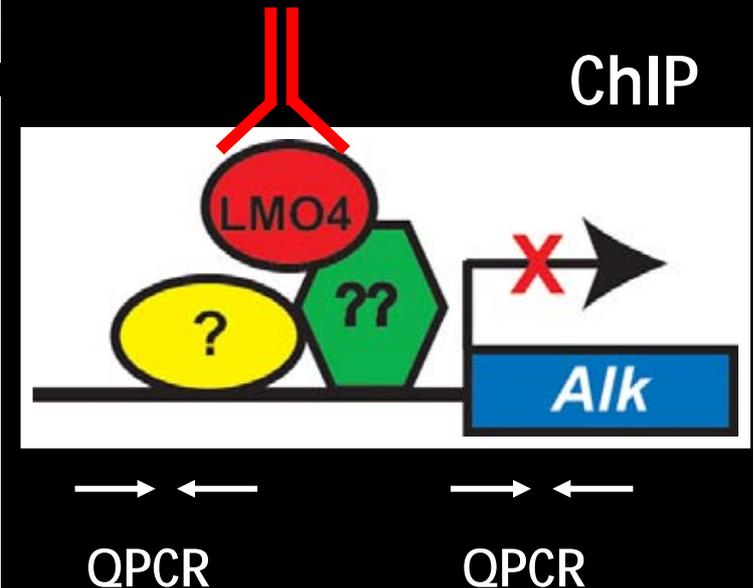
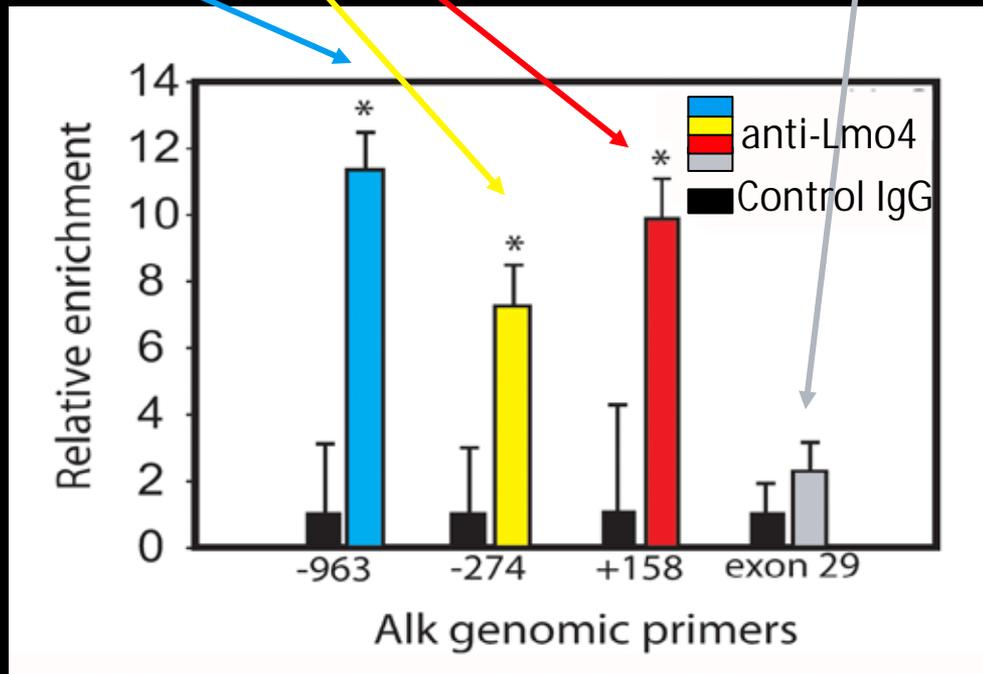
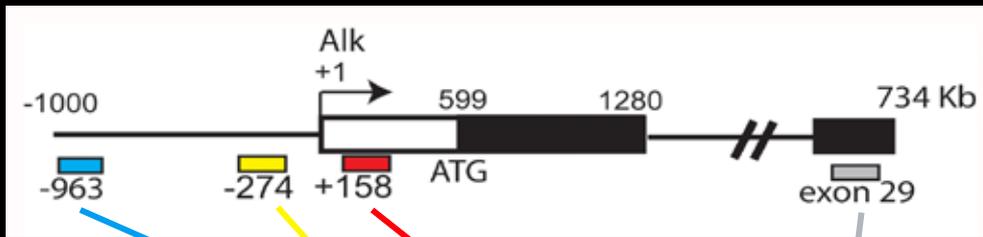
Lmo4 function in the **adult Acb** to regulate the acquisition and maintenance of cocaine sensitization

Does *Lmo4* inhibit *Alk* expression in *Acb* ?

LCM-qPCR



Does LMO4 regulate *Alk* expression directly?



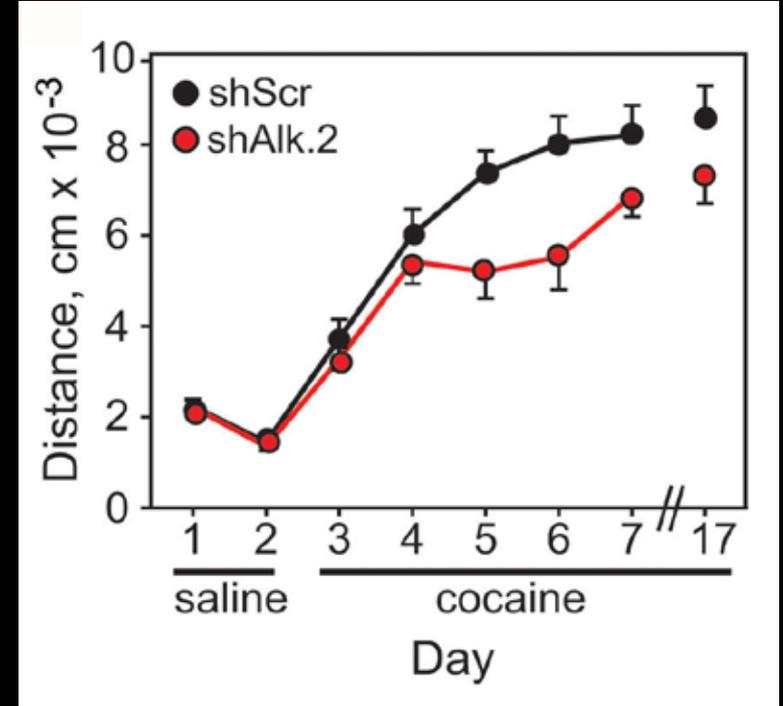
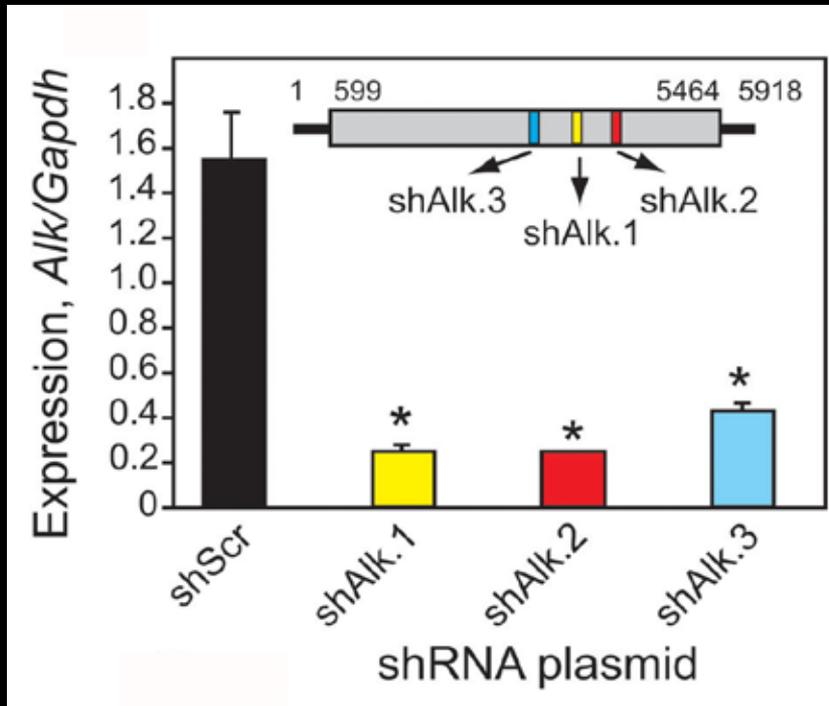
LMO4 is associated with *Alk* promoter in striatum, which is expected to inhibit *Alk* expression.

Is *Alk* involved in cocaine sensitization?



Prediction: Down-regulation of *Alk* should reduce/impair cocaine sensitization

Alk downregulation in Acb...

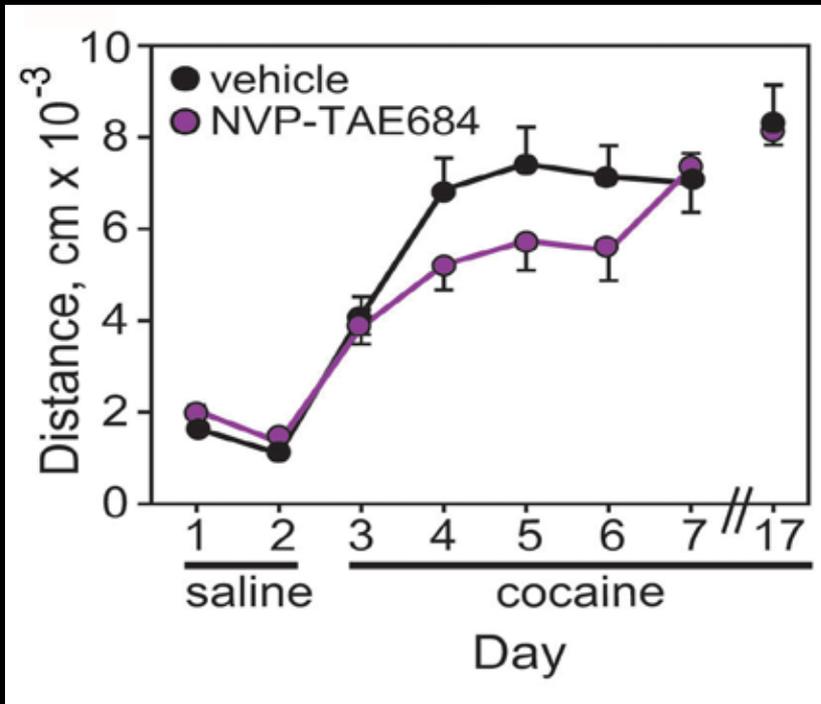


... reduces rate of acquisition of cocaine sensitization

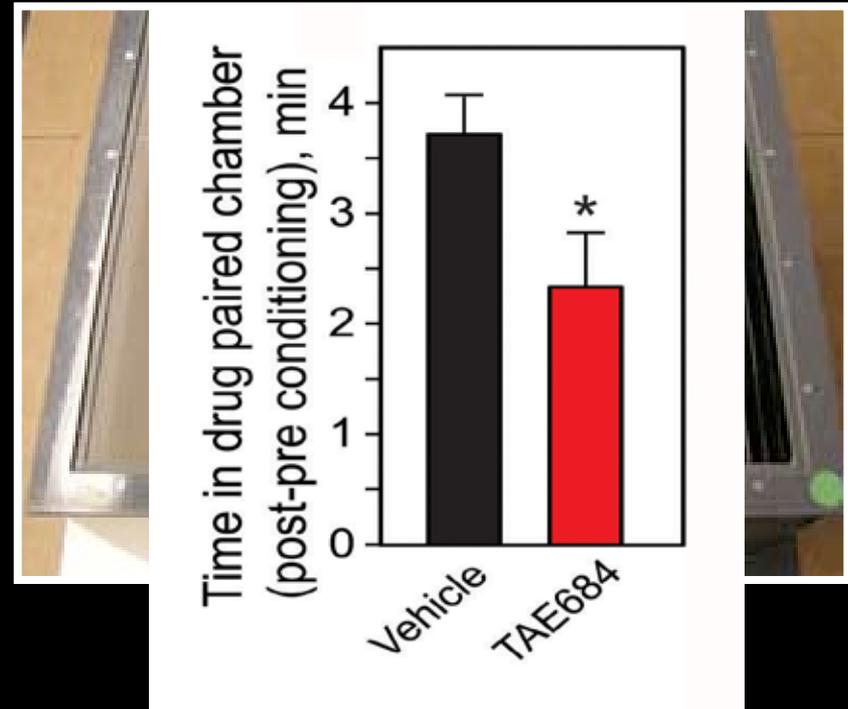
LMO4-ALK... does it matter?



Cocaine sensitization



Cocaine CPP



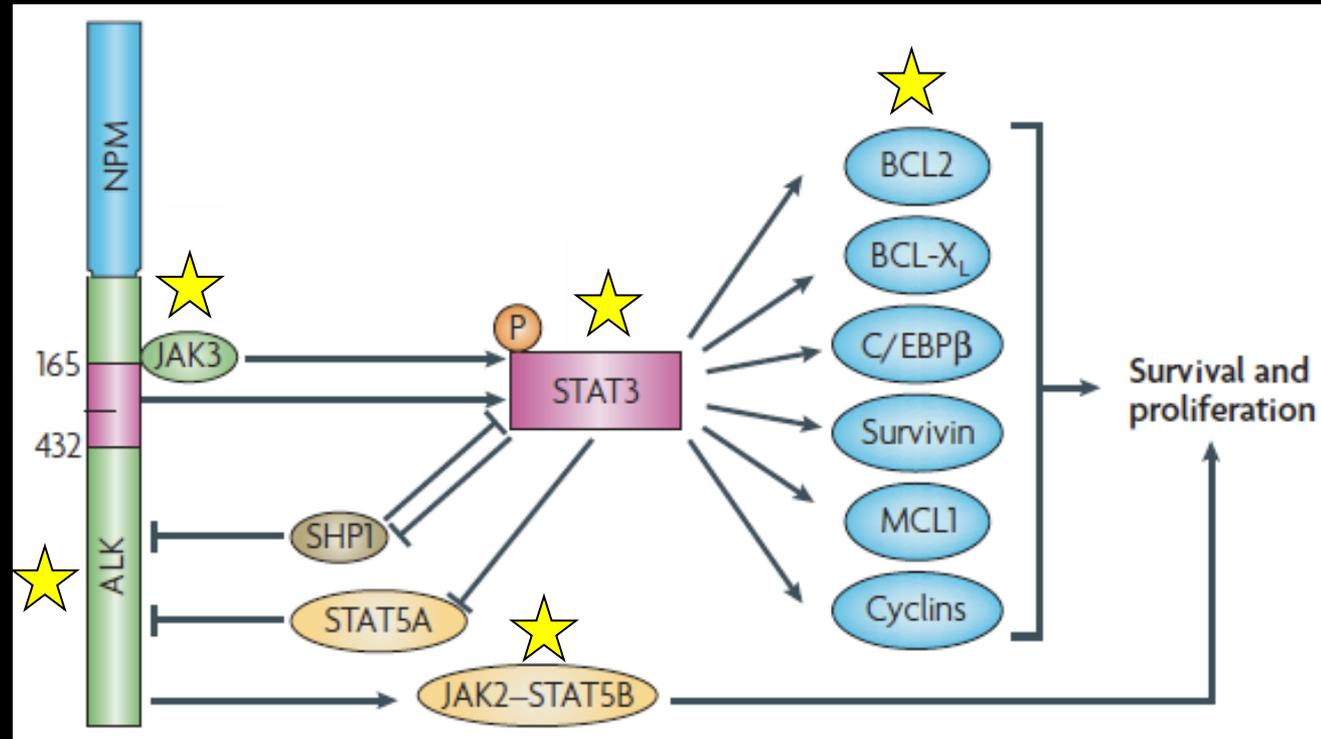
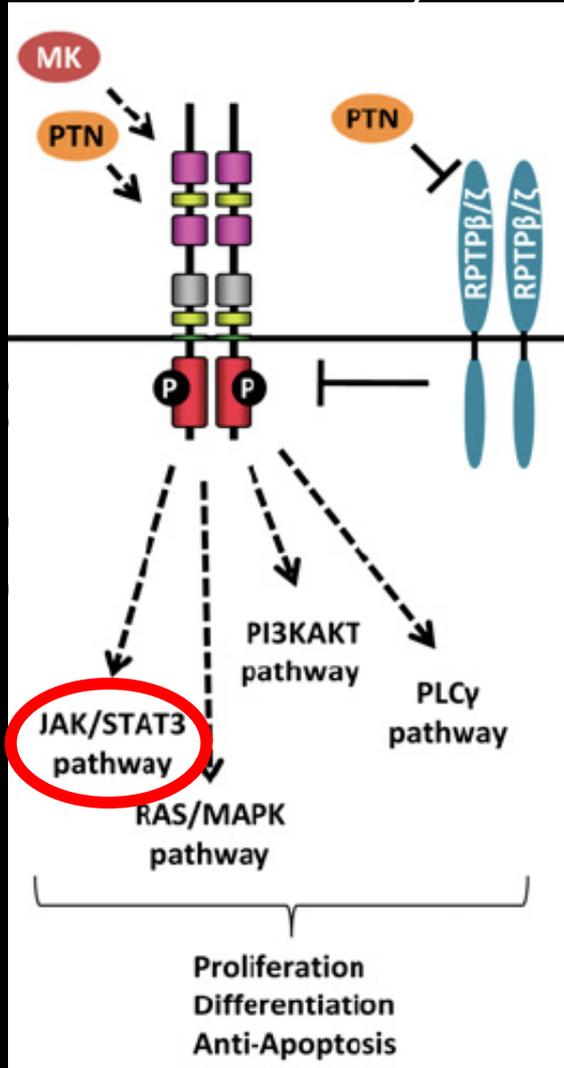
Acute inhibition of ALK delays cocaine sensitization and reduces the rewarding properties of cocaine

ALK inhibitors are in development for cancer, ALK may therefore be a novel therapeutic target for addiction.

Amy Lasek (unpublished)

Not just ALK.... but its signaling pathways!

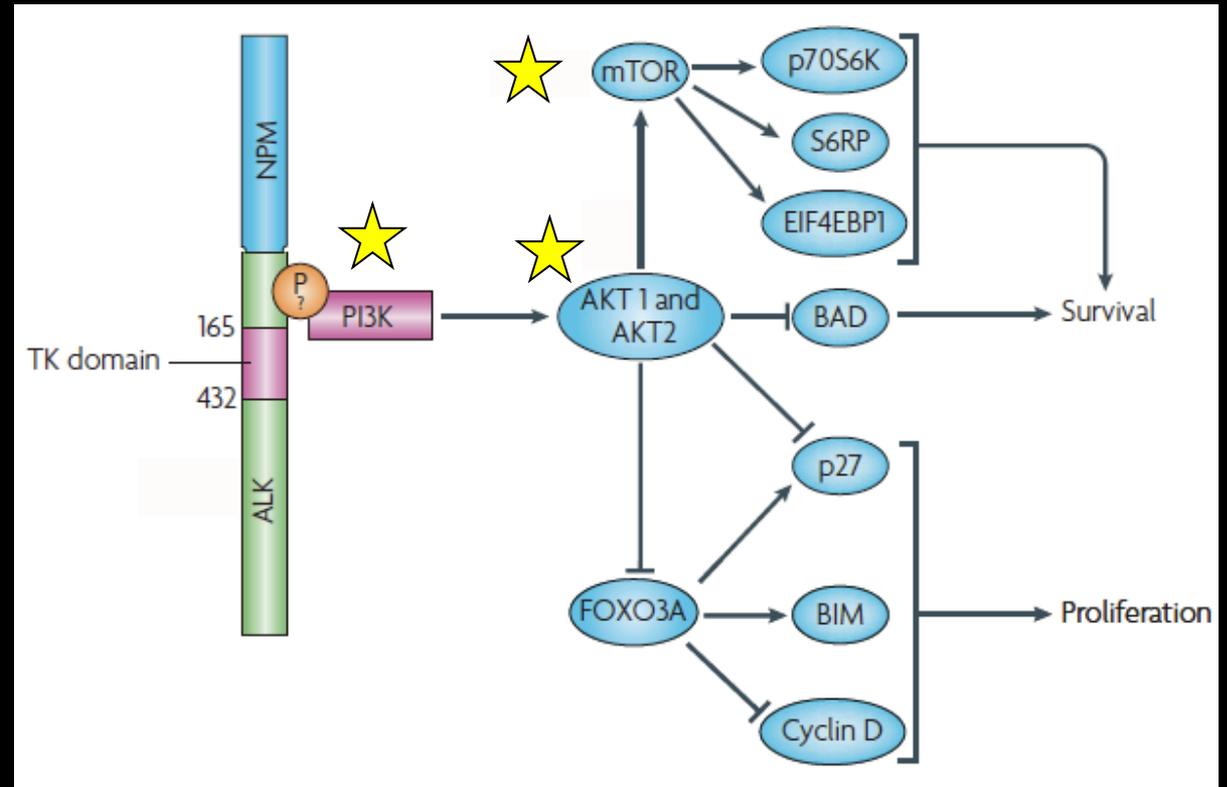
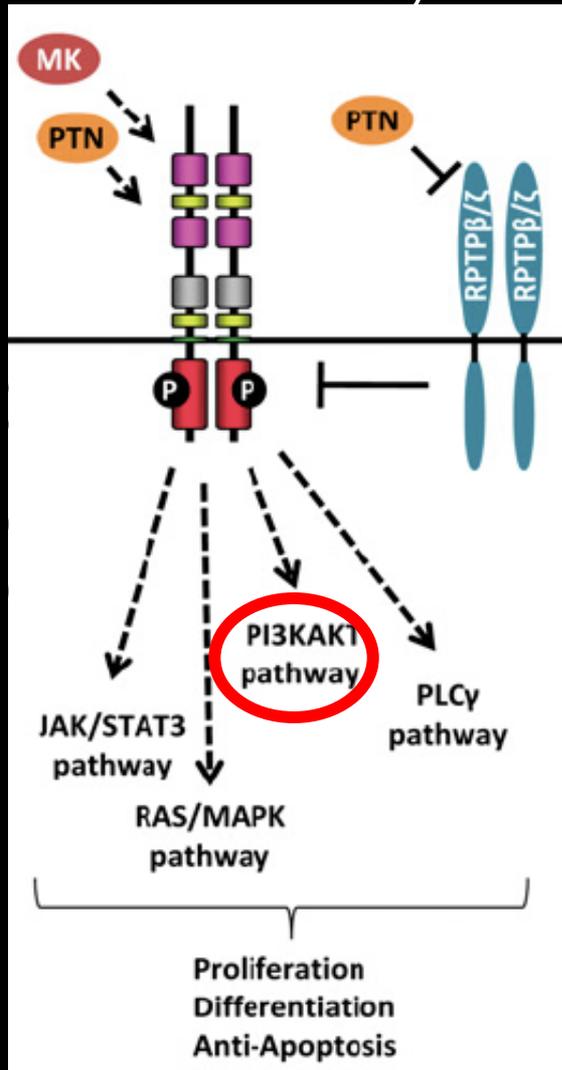
ALK Pathway



Pharmacotherapy available / in development

Not just ALK.... but its signaling pathways!

ALK Pathway



Pharmacotherapy available / in development

SUMMARY

Validation of *Lmo4*
in cocaine sensitivity
and sensitization

Alk regulated by
LMO4

dAlk RTK
regulated by dLMO

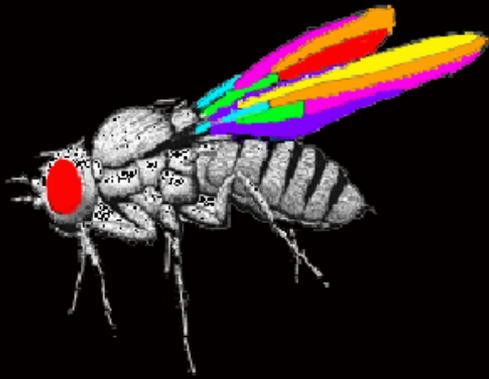
Alk regulates cocaine
sensitization

dLmo mutants

Cocaine sensitivity
screen

ALK small-molecule inhibitor
reduces cocaine sensitization
and reward







Roland Bainton

Linus Tsai



Linus Tsai
Roland Bainton



The mouse team (EGCRC)



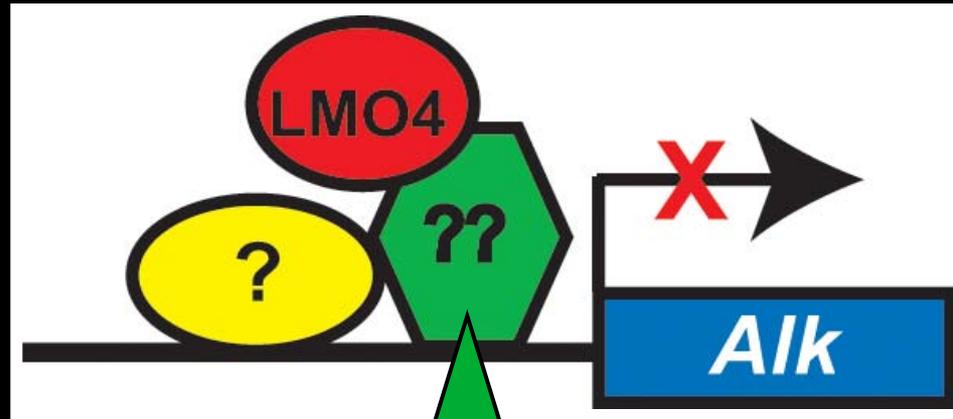
Amy Lasek

Francesco Giorgetti

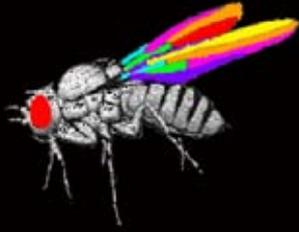
David Kapfhamer

Julie Gesch

How does LMO4 regulate *Alk* expression??



Poster by Amy Lasek



Marco Milan, Steve Cohen,
Nick Justice, Yuh-Nung Jan



Steve Morris (St. Jude's)
EGCRC Animal Core Facility
EGCRC Histology Core Facility



NIH/NIDA
State of California
Department of Defense

The image features a dense grid of small, colorful illustrations of various insects, including beetles, flies, and bees, set against a black background. The insects are rendered in a variety of colors such as red, blue, green, yellow, and purple. In the center of the grid, the text "Thank you!" is written in a large, white, sans-serif font.

Thank you!